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Repair of full-thickness cartilage defects using liposomal transforming growth factor-β1

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Abstract Transforming growth factor-β1 (TGFβ1) is a well-known, potent growth factor implicated in both in vitro and in vivo chondrogenesis. Liposomes have been employed as a drug delivery system to promote the efficient use of drugs. The objective of this study was to demonstrate that a single injection of liposomal TGFβ1 has an accelerating effect on the repair of an articular cartilage defect. Full-thickness articular cartilage defects were prepared on the patellar grooves of the femurs in knee joints of Japanese white rabbits. One week after surgery, various reagents including liposomal TGFβ1, free TGFβ1, and phosphate-buffered saline were injected into the operated knee joints. At 3 weeks after surgery the specimens obtained from the lesions were evaluated histologically, and the glycosaminoglycan content was quantified. Histological examination revealed that the defects were filled with thicker fibrous cartilage and showed more intense metachromatic staining in the liposomal TGFβ1 group than in the other groups. The glycosaminoglycan content of the repair tissue was also significantly higher in the liposomal TGFβ1 group than in the other groups. This study indicated that the intraarticular injection of liposomal TGFβ1 could accelerate the early-stage repair of full-thickness articular cartilage defects.

Key words Transforming growth factor (TGF)-β1 · Full-thickness articular cartilage defects · Intraarticular injection · Liposomes

Introduction

Articular cartilage is an important constituent of joints with various functions, including lubrication and absorption of shocks. Injury or destruction of the articular cartilage results in deterioration of the joint. Accordingly, repairing such injured articular cartilage is extremely important to maintain adequate articular function. The repair of injured cartilage to a state that is as perfect as that of hyaline cartilage has been considered impossible. However, Brittberg et al. reported that when they implanted incubated autogenous cartilage cells at the site of a full-thickness cartilage defect the cartilage was restored to a condition that was histologically similar to the original hyaline cartilage.[3] A variety of attempts have also been made in basic and clinical settings to develop cultured chondrocytes and media that could be used to fill and repair cartilage defects.[4]

Many attempts have been made to accelerate the repair of articular cartilage injuries using non-invasive methods. These attempts have included single or repeated intraarticular injection of reagents that may promote restoration of the cartilage, such as growth factors. In particular, fibroblast growth factor-2 (FGF-2) has long been suggested as a strong mitogen for in vitro chondrogenesis. In a 1980 in vivo study, Wellmitz et al. injected FGF-2 intraarticularly into a joint with a cartilage defect in a rabbit model and found that FGF-2 effectively promoted cartilage restoration in both full-thickness and partial defects.[28] FGF-2 has subsequently been studied at other institutions and is presently regarded as the most effective growth factor available for cartilage restoration.[5,13] Insulin-like growth factor (IGF) has also been reported to be involved in chondrogenesis.[25] In canine osteoarthritis models, Rogachefsky et al. found that intramuscular injection of sodium pentosan polysulfate (PPS) and IGF was effective therapy for osteoarthritis.[20] Wakitani et al. made repeated intraarticular injections of hepatocyte growth factor (HGF), which has diverse biological activities, into full-thickness cartilage defects in a rabbit model, monitored a long-term (24 weeks) course, and reported a positive effect on restoration.[27] Among these various

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growth factors, transforming growth factor-β1 (TGFβ1) has been suggested to play an important role in in vitro chondrogenesis. Glansbeek et al. reported that intra-articular injections of TGFβ1 stimulated the replenishment of proteoglycans in arthritis models.[9] However, intraarticular injection of TGFβ1 has never been assessed using cartilage defect models.

Liposomes have been employed as a drug delivery system to promote the efficient use of drugs.[18] The liposomes serve as carriers, allowing improved drug retention and reduced toxicity. They have also been applied in the field of gene therapy. Our former study showed that fluorescence (DiI) accumulated at the site of cartilage repair after intraarticular injection of fluorescence-labeled liposomes.[1] This observation suggested the possibility that the drug encapsulated into liposome had some positive effects on in vivo cartilage restoration.

We believe that efficient techniques for delivering drugs to promote the restoration of cartilage injuries are required to enable injured cartilage to be repaired noninvasively. In this study, we demonstrated that articular cartilage defects could be significantly restored after a single intraarticular injection of TGFβ1 using a liposome drug delivery system.

Materials and methods

Preparation of full-thickness cartilage defects

A total of 53 female Japanese white rabbits (2–3 months of age) (Tokyo Laboratory Animals Science, Tokyo, Japan), each weighing 2.5 kg, were used in this study. The rabbits were anesthetized intravenously with sodium pentobarbital (25 mg/kg). Following a medial parapatellar incision, the patella was dislocated laterally. A full-thickness defect (6 mm long × 3 mm wide) was created using a hand drill with a drill bit (3 mm in diameter) and a scalpel, which were passed through the articular cartilage into the subchondral bone of the patellar groove of the femur in the left knee joint. The depth of the defects was determined using a depth gauge; all defects were a uniform 3 mm in depth. The joints were irrigated with saline solution to remove any free cartilage and bone fragments. The joint capsule and skin were then sutured with monofilament nylon thread. Immobilization was not applied, and the rabbits were allowed to move freely in their cages after the surgery.

All rabbits were killed by intravenous administration of sodium pentobarbital 75 mg/kg. Altogether, 47 rabbits were used for histological evaluation or quantification of glycosaminoglycan (GAG) content. Six were used for histological evaluation of the expression of type II collagen by immunohistochemistry and in situ hybridization.

All surgical interventions were performed under sterile conditions. The rabbits were housed and maintained in accordance with the guidelines established by the National Institutes of Health Sciences.

Preparation of liposomes containing TGFβ1

Liposomes were prepared from a lipid mixture of egg yolk phosphatidylcholine (egg-PC, 5 μmol), cholesterol (Ch, 5 μmol), and dipalmitoylphosphatidic acid (DPPA, 0.5 μmol). The liposomes were negatively charged. The dried lipid film, obtained by rotary evaporation and subsequent vacuum desiccation, was dispersed in 0.1 or 0.2 ml of phosphate-buffered saline solution (PBS), with or without human recombinant TGFβ1 (Amersham, Buckinghamshire, UK) at a dose of 100 ng. When using these lipids, the rate of drugs trapped within the liposomes ranged from 10.3% to 16.2% (average 12.9%). The rate was determined using carcein (Wako, Tokyo, Japan), as described by Oku et al.[17]

Intraarticular injection of drugs

The liposomal solution (0.2 ml) was resuspended in saline containing 100 ng TGFβ1. One week after surgery this liposomal solution was injected into the surgically treated knee joints (lipo-TGF group). For the controls, PBS solution (PBS group), liposomal PBS solution without TGFβ1 (lipo-PBS group), and PBS solution containing 100 ng TGFβ1 (TGF group) were injected into the operated knee joints. Furthermore, a mixture of PBS solution (0.1 ml) with TGFβ1 (100 ng) and liposomal PBS solution (0.1 ml) without TGFβ1 was injected into the knee as a control in which TGFβ1 was present but not encapsuled by the liposomes (non-lipo-TGF group). A preliminary experiment showed that cartilage restoration was optimal in the liposomal TGFβ1 100 ng group compared to the liposomal TGFβ1 10 ng and 500 ng groups. A concentration of 100 ng TGFβ1 was thus used as the most suitable dosage for cartilage repair in the present experiment.

These drugs and the short-term groups (3 weeks after surgery) are shown in Table 1 (each group: $n = 7$). The histological evaluations of the long-term (12 weeks after surgery) were subsequently examined in the PBS, TGF, and lipo-TGF groups (each group: $n = 4$).

Histological evaluation

The knee joint was dissected, and the patellar groove of the femur was examined histologically. The proximal half of the repair tissue was used for histological

Table 1. Groups of rabbits in the experiment

Group	Volume of TGF-β1 (ng)	Liposomes	Free TGF-β1	Encapsuled TGF-β1 in liposomes
PBS	0	−	−	−
Lipo-PBS	0	+	−	−
TGF	100	−	+	−
Lipo-TGF	100	+	+	+
Nonlipo-TGF	100	+	+	−

At 1 week after surgery, PBS containing these drugs was injected into the left knees of the rabbits
PBS, phosphate-buffered soline; Lipo, liposome; TGF, transforming growth factor; +, drug contained TGFβ1, liposomes, and TGFβ1 encapsulated in liposomes; −, drug contained none of the aforementioned substance

evaluation, and the distal half was used for quantification of the GAG content. The specimens from the distal part of the femur were fixed in 10% buffered formalin for 1 week. Each specimen was decalcified using Plank-Rychlo solution for 5 days, embedded in paraffin, cut into coronal sections, and then stained with toluidine blue. The sections were examined microscopically and scored according to the histological scoring method described by Wakitani et al.[26] (Table 2). The scoring data were "blindly" evaluated by the authors (T.A., H.N., T.K.).

Table 2. Wakitani's histological scoring

Category	Points
Cell morphology	
Hyaline cartilage	0
Mostly hyaline cartilage	1
Mostly fibrocartilage	2
Mostly noncartilage	3
Noncartilage only	4
Matrix staining (metachromasia)	
Normal (compared with host adjacent cartilage)	0
Slightly reduced	1
Markedly reduced	2
No metachromatic stain	3
Surface regularity	
Smooth (>3/4)	0
Moderate (>1/2–3/4)	1
Irregular (1/4–1/2)	2
Severely irregular (<1/4)	3
Thickness of cartilage	
>2/3	0
1/3–2/3	1
<1/3	2
Integration of donor with host adjacent cartilage	
Both edges integrated	0
One edge integrated	1
Neither edge integrated	2
Maximum total	14

This table was extracted from Wakitani et al.'s histological grading scale[26]

Quantification of GAG content

The GAG content was measured quantitatively using a 1,9-dimethylmethylene blue (DMB) assay and a fluorometric DNA assay. Papain (Sigma, St. Louis, MO, USA) was dissolved at a concentration of 300 μg/ml in a 50 mM phosphate buffer (pH 6.5) with 5 mM *N*-acetylcysteine and 2 mM ethylenediaminetetraacetic acid (EDTA). After carefully sampling the distal part of the repaired tissue (from the superficial layer to the deep layer, without any subchondral bone or surrounding cartilage), the samples were immediately digested in a papain solution at 60°C for 16 h.

A DMB assay was performed using the method described previously by Goldberg and Kolibas.[10] Briefly, 140 μl of the sample was mixed 1:1 with DMB solution (Polysciences, Warrington, PA, USA) in a 96-well microtiter plate. The absorbance (530 nm) was measured using a Titertek multiscan spectrophotometer (Labsystems, Helsinki, Finland). Standard curves were generated using a previously established concentration of chondroitin sulfate C (Seikagaku, Tokyo, Japan).

A fluorometric DNA assay of the samples was performed using the method described previously by Kim et al.[14] In a 96-well microtiter plate, 15 μl of the papain-digested explants were mixed with 300 μl of Hoechst 33258 dye (Polysciences). The emission and excitation spectra were determined at 365 nm and 458 nm, respectively. The fluorescence emission was measured using a Titertek multiscan spectrofluorometer. Standard curves were generated at a known concentration of calf thymus DNA (Sigma).

Immunohistochemistry of type II collagen

Tissue specimens for immunohistochemistry and in situ hybridization of type II collagen mRNA were obtained from the PBS and lipo-TGF groups 3 weeks after surgery (each group, $n = 3$). After being killed, the specimens were immediately fixed in 4% paraformaldehyde (Wako, Osaka, Japan) for 1 week. Each specimen was decalcified using 0.5 M EDTA (pH 7.4)

for 4 weeks, embedded in paraffin, and then cut into coronal sections.

The immunohistochemistry was performed as described by Nakajima et al.[16] The specific sections were deparaffinized and pretreated enzymatically with actinase E 0.1 mg/ml (Kaken, Tokyo, Japan) in PBS at 37°C for 30 min. The degree of nonspecific background staining was reduced by incubating the sections with a 1:50 mixture of normal horse serum in PBS at room temperature for 60 min. The sections were incubated overnight with mouse anti-human type II collagen monoclonal antibody (50 μg/ml in PBS containing 0.1% bovine serum albumin) (Fuji, Takaoka, Japan) at 4°C. The sections were then incubated with horse anti-mouse biotinylated immunoglobulin G (IgG) diluted 1:200 with PBS (Vector Laboratories, Burlingame, CA, USA) for 60 min and with fluorescein streptavidine diluted 1:100 with PBS (Vector) at room temperature for 45 min.

In situ hybridization of type II collagen

Linearized vectors containing a fragment of the type II procollagen C-peptide region were created using the method reported by Takaishi et al.[24] For the in situ hybridization, digoxygenin-labeled antisense or sense transcripts were generated from the linearized vectors by in vitro transcription using T7 or SP6 RNA polymerase and an RNA labeling kit (Boehringer-Mannheim, Mannheim, Germany). After transcription the DNA templates were removed by DNase treatment, and the labeled RNA transcripts were recovered after phenol-chloroform extraction and precipitation with ethanol prior to storage at −80°C.

The sections were treated with proteinase K (20 μg/ml) at room temperature for 20 min, refixed in 4% paraformaldehyde, acetylated with acetic anhydride (0.25% in 0.1 M triethanolamine), washed again in PBS and distilled water, and then rehydrated. The sections were hybridized with antisense or sense riboprobes at 50°C for 16 h. The hybridization buffer contained 50% formamide, 10% dextran sulfate, 10 mM dithiothreitol, 200 μg/ml tRNA, and Denhardt's solution (×1) in a humidified chamber. After hybridization, the tissue sections were washed in saline and sodium citrate buffer (SSC) (×1)/50% formamide for 20 min at 45°C and then treated with RNase A 2 μg/ml (in 10 mM Tris-HCl, 0.5 M NaCl, and 1 mM EDTA) at 37°C for 30 min. They were then washed in SSC (×2) and SSC (×0.2), each at 37°C for 20 min. The immunological detection system, consisting of antibody conjugate and nitroblue tetrazolium chloride, was used according to the manufacturer's instructions (Boehringer-Mannheim).

Statistical analysis

The histological scores and GAG content were analyzed using the *F*-test. When the variance was equal, the data were further analyzed accordingly to Fisher's protected least significant difference. The difference was considered significant if $P < 0.05$.

Results

Histological findings

No signs of arthritis, such as osteophytes or severe synovial proliferation, were observed in any of the treated groups. Toluidine blue staining showed that the cartilage defects in six knees of the PBS group were filled with a thin, fibrous tissue that was involved in the convex and irregular surface and showed no or poor metachromasia. The nonintegrated gap between the surrounding cartilage and the repair tissue was visible (Fig. 1A). Under high-power magnification, the superficial and middle layers of the repair tissue showed an irregular extracellular matrix involving spindle-shaped cells resembling undifferentiated mesenchymal cells (Fig. 1A′); the deep layer partially showed a fine extracellular matrix involving round, immature chondrocyte-like cells. Most specimens in the TGF group, the lipo-PBS group, and the non-lipo-TGF group also showed immature repair tissue similar to that seen in the PBS group.

On the other hand, in five specimens of the lipo-TGF group the defects were filled with a thick, relatively smooth-surfaced repair tissue resembling fibrocartilaginous tissue and also occasionally showed intense metachromasia in the middle and deep layers (Fig. 1B). The histological difference between the surrounding cartilage and the repair tissue was significantly recognizable. No tide marks were observed in any of the specimens. Under high-power magnification, the superficial layer of the repair tissue showed an irregular extracellular matrix and was involved with spindle-shaped cells resembling undifferentiated mesenchymal cells. However, the middle and deep layers showed a fine extracellular matrix resembling fibrous cartilage and were involved with round, relatively mature chondrocyte-like cells (Fig. 1B′).

Histological scoring of repair tissue

The mean scores obtained using the Wakitani's histological scoring method are shown in Fig. 2. The total score for the lipo-TGF group was significantly better than the scores of all other groups ($P < 0.05$). No significant differences were observed among the other groups, except for the lipo-TGF group. According to

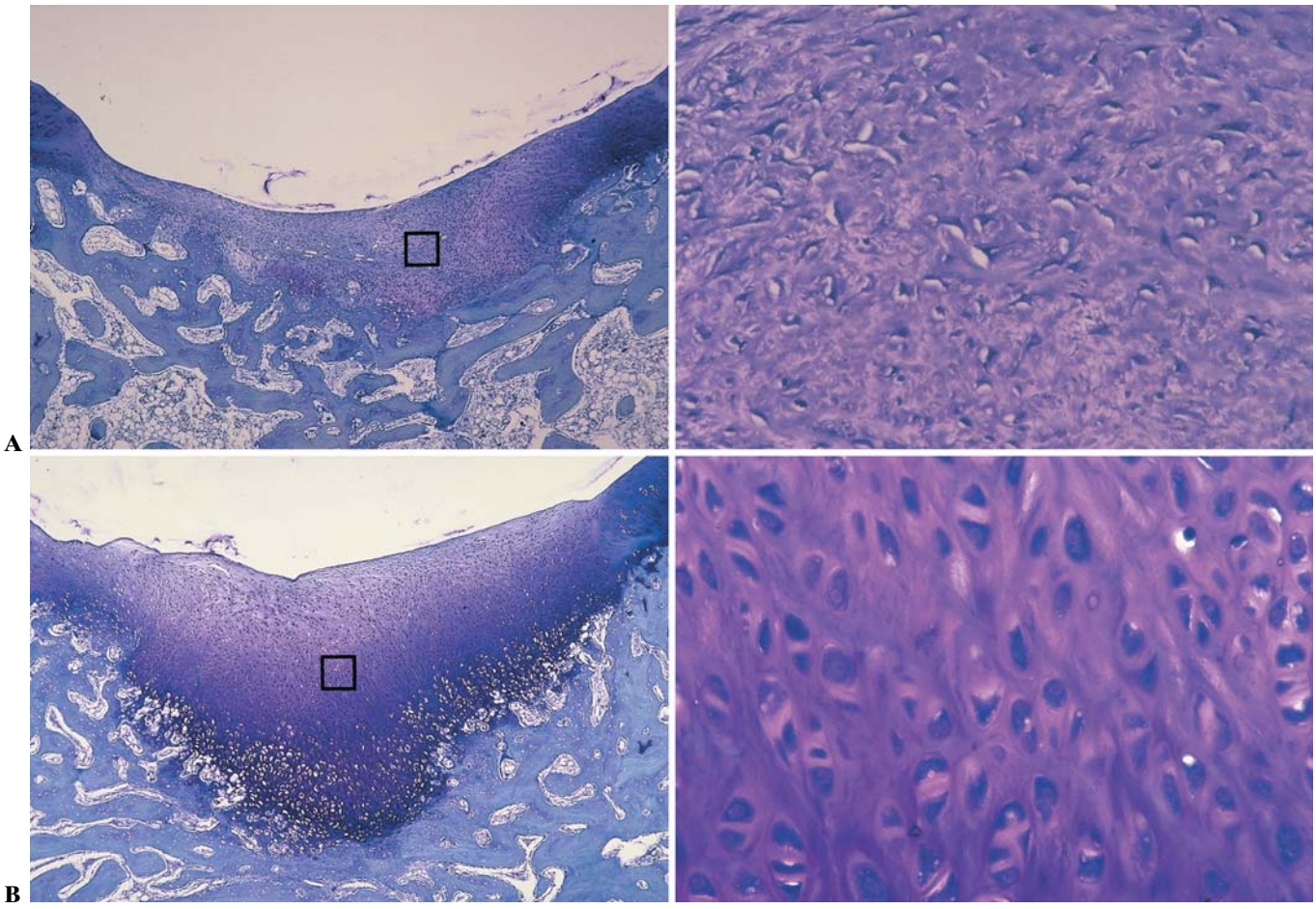

Fig. 1. Histological findings 3 weeks after surgery using toluidine blue staining. **A** Phosphate-buffered saline (PBS) group. Cartilage defects were filled with thin, fibrous tissue with an irregular convex surface; they showed no or poor metachromasia. The nonintegrated gap between the surrounding cartilage and the repair tissue was visible. (×10) **B** Liposomal solution containing transforming growth factor (lipo-TGF) group. The defects were filled with a thick and relatively smooth-surfaced repair tissue resembling fibrocartilaginous tissue; it also occasionally showed intense metachromasia in the middle and deep layers. (×150) **A,B** The middle layer in the central portion of the repair tissue is shown in the *squares*. (*A′*), PBS group. Middle layers of the repair tissue had an irregular extracellular matrix and were involved with spindle-shaped cells resembling immature mesenchymal cells. (*B′*), lipo-TGF group. Middle layers of the lipo-TGF group had a fine extracellular matrix resembling fibrous cartilage and were involved with round, relatively mature chondrocyte-like cells

the five categories evaluated by Wakitani's scoring system, a significant difference was observed only in the thickness scoring between the lipo-TGF group and all other treated groups ($P < 0.05$). Moreover, significant differences were observed in the morphology and metachromasia between the lipo-TGF group and the other treated groups ($P < 0.05$), except for the lipo-PBS group (morphology $P = 0.1649$, metachromasia $P = 0.0997$).

GAG content of the repair tissue

The GAG content of the repair tissue was significantly higher in the lipo-TGF group than in all other groups ($P < 0.05$) (Fig. 3). No significant differences were observed among the other groups.

Immunohistochemistry of type II collagen and in situ hybridization of type II procollagen mRNA

In an immunohistochemical analysis of type II collagen, all specimens in the PBS group and the lipo-TGF group showed no or weak staining in the central region of the superficial and middle layers (Fig. 4A,B). Only weak staining was observed in the extracellular matrix in the deep layer of the central portion of the PBS group (Fig. 4A′). On the other hand, immunohistochemical assessment of the lipo-TGF group revealed intense

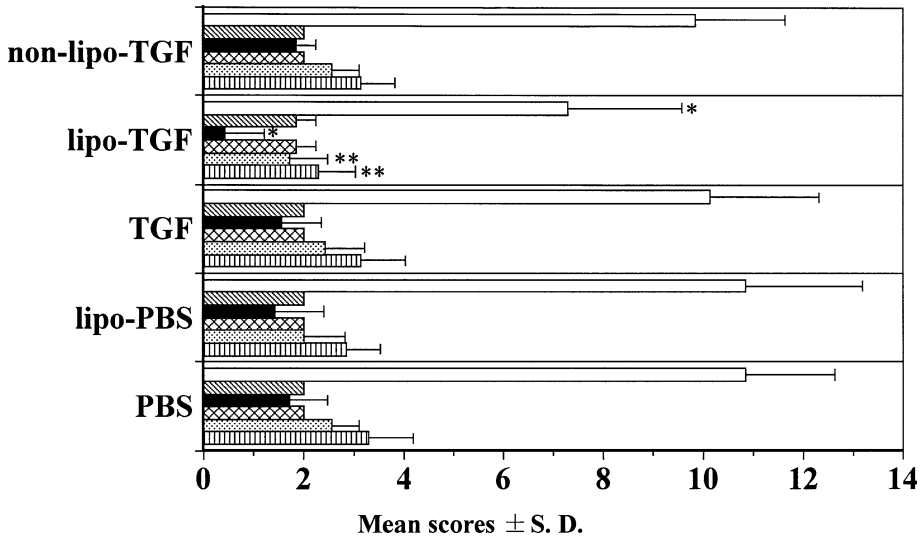

Fig. 2. Scoring for repair tissue 3 weeks after surgery. The mean scores were obtained using the histological scoring system described by Wakitani et al.[26] Significant differences between the lipo-TGF group and the other treated groups are shown for the total scores (*$P < 0.05$ vs. all other groups). A significant difference in thickness was observed between the lipo-TGF group and the other treated groups (*$P < 0.05$ vs. all other groups). Significant differences in morphology and metachromasia were also observed between the lipo-TGF group and all other treatment groups, except for the lipo-PBS group (**$P < 0.05$ vs. TGF, nonlipo-TGF, and PBS groups)

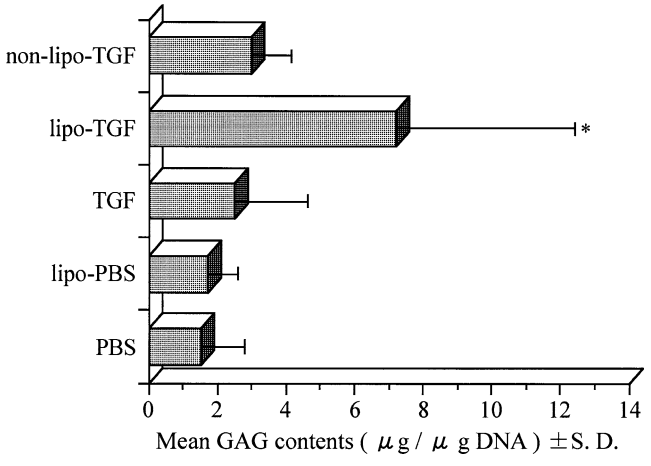

Fig. 3. Glucosaminoglycan (GAG) content of the repair tissue 3 weeks after surgery. A significant difference was observed between the lipo-TGF group and the other treatment groups (*$P < 0.05$ vs. all other groups)

staining of type II collagen only in the deep layer (Fig. 4B′).

During in situ hybridization of type II procollagen mRNA, no expression was observed in any cells of the superficial or middle layer of the PBS group (Fig. 5A). The PBS group showed weak signals only in the cells of the deep layer, suggesting that these cells may be chondrocytes or prechondrocytes (Fig. 5A′). On the other hand, samples from the lipo-TGF group showed positive expression in many cells of all layers, ranging from undifferentiated mesenchymal cells in the superficial layer to chondrocyte-like cells in the deep layer (Fig. 5B,B′).

Evaluation 12 weeks after surgery

The cartilage defects in two or three specimens in all groups (PBS, TGF, and lipo-TGF groups) were filled with a thick, irregular-surfaced repair tissue involved with round chondrocyte-like cells, resembling fibrocartilaginous tissue. In two or three knees in the PBS and TGF groups, the repair tissue showed intense metachromasia only in the deep layer and an irregular extracellular matrix. On the other hand, in three specimens in the lipo-TGF group, the repair tissue showed intense metachromasia in the middle and deep layers and a fine extracellular matrix. The nonintegrated gap between the surrounding cartilage and the repair tissue was visible in all groups (Fig. 6).

Discussion

Transforming growth factor-β1 is known to promote differentiation of mesenchymal stem cells into chondrocytes and to facilitate in vitro synthesis of matrix molecules, such as collagen, proteoglycan, and fibronectin.[12,19,21,22] Therefore, TGFβ1 has long been proposed as a potential promoter of cartilage restoration. Hunziker and Rosenberg recently reported that they filled partial cartilage defects in the knee joints of rabbits using fibrin glue containing TGFβ1 liposomes and succeeded in restoring hyaline cartilage.[11] There have been no detailed reports on the effects of the intraarticular injection of TGFβ1 with regard to cartilage restoration in full-thickness defects.

The present study is the first demonstration of a noninvasive treatment using TGFβ1 for cartilage restoration in full-thickness defects. We demonstrated in vivo that only TGFβ1 encapsulated into liposomes

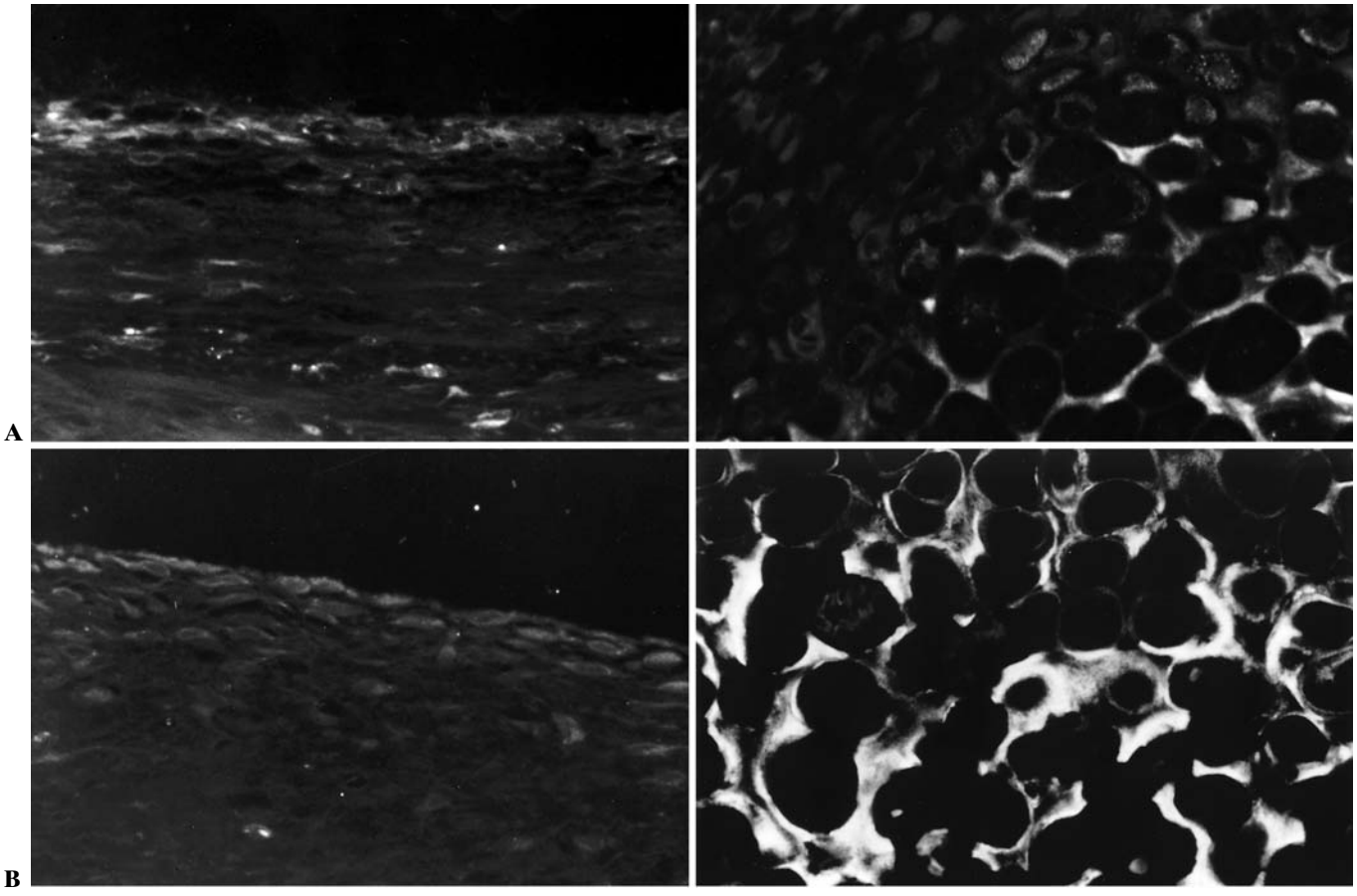

Fig. 4. Immunohistochemical staining of type II collagen in the specimens from the PBS group and the lipo-TGF group 3 weeks after surgery. **A** Superficial and middle layers of the PBS group showed no or weak staining in the central region of the superficial and middle layers. **B** Superficial and middle layers of the lipo-TGF group also showed no or weak staining. **A′** Deep layer of the PBS group showed weak staining in the extracellular matrix. **B′** Deep layer of the lipo-TGF group showed intense staining in the extracellular matrix. **A–B′** ×200

has the ability to promote articular cartilage restoration. This promoting effect was quantitatively reflected by improving Wakitani's histological score; the total score of the lipo-TGF group, which was treated using liposome-encapsulated TGFβ1, was significantly better than the scores of the other groups. In particular, the scores for metachromasia and cell morphology were better in the lipo-TGF group than in the other groups. The quantitative increase in the proteoglycan contents was also verified using the DMB method. Moreover, histologically, the repair tissue in most of the lipo-TGF specimens was thicker and contained many round chondrocyte-like cells in the middle and deep layers. These observations suggest that cartilage regeneration may be accelerated by the liposome-encapsulated TGFβ1. The patterns of positive immunostaining and mRNA expression of type II collagen were recognized more often in the lipo-TGF group than in the PBS group. The positive immunostaining pattern and the mRNA expression of type II collagen in the deep layer, which is adjacent to the bone marrow, seem to indicate that liposome-encapsulated TGFβ1 activates migration of the mesenchymal cells from the bone marrow and their differentiation into chondrocytes, facilitating the synthesis of major matrix molecules such as proteoglycan and type II collagen. Our in vivo results showing positive immunostaining and the mRNA expression of type II collagen support the idea that TGFβ1 has the ability to facilitate synthesis of type II collagen in vitro.

The results of this study appear to be attributable to some of the advantages of using liposomes as the drug delivery system for cartilage restoration. The drug retention time in joints has been reported to increase considerably when drugs are encapsulated in liposomes. Bard et al. reported that the duration of ^{51}Cr retention in joints intraarticularly injected with ^{51}Cr encapsulated in liposomes was longer than when ^{51}Cr was injected straight into the joints.[2] Foong and Green reported that

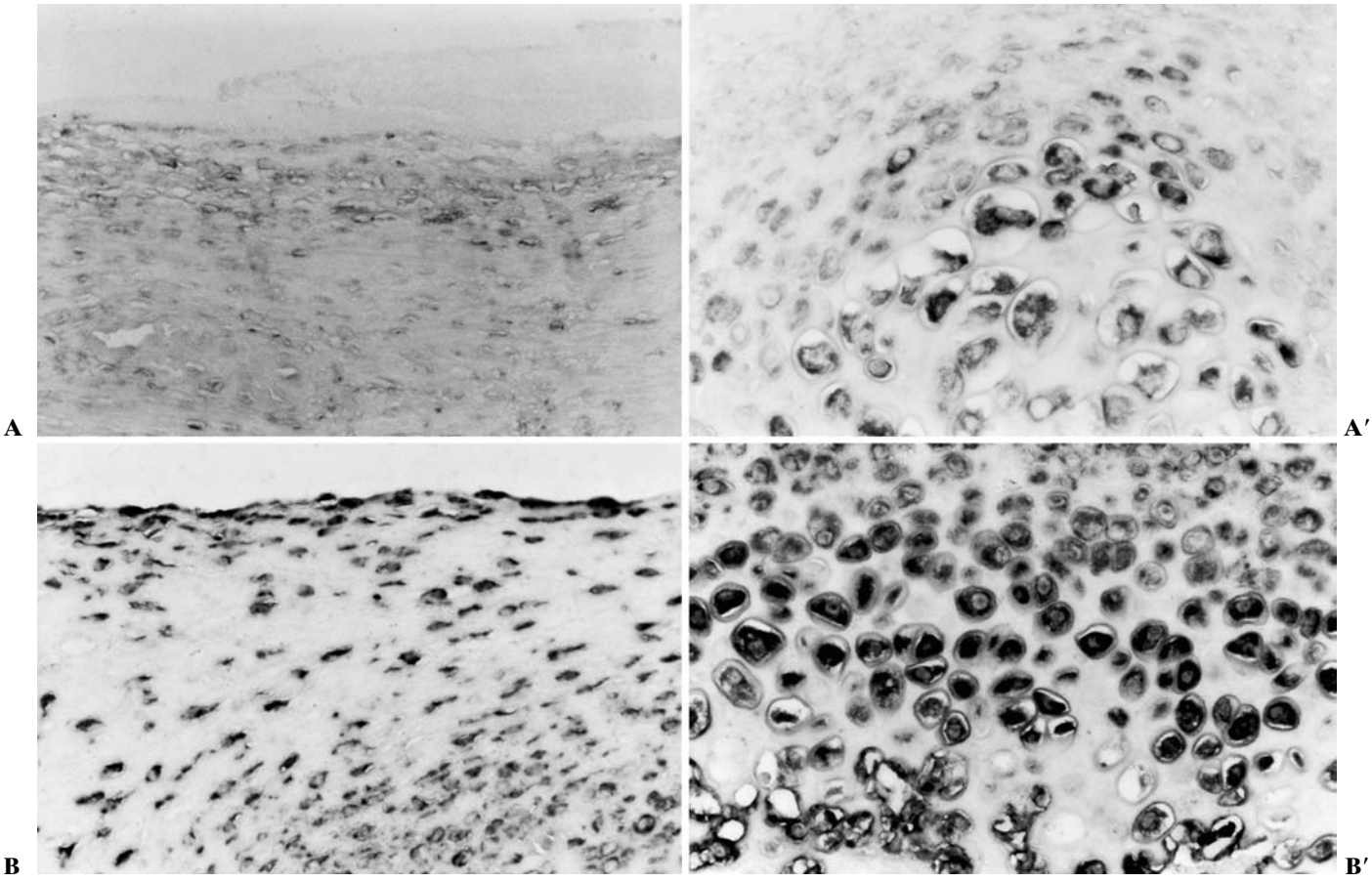

Fig. 5. In situ hybridization of type II collagen mRNA 3 weeks after surgery. **A** Superficial and middle layers of the PBS group: no expression in any cells. **B** Superficial and middle layers of the lipo-TGF group: positive expression in many undifferentiated mesenchymal cells in the superficial layer. **A′** Deep layer of the PBS group: weak signals only in the cells of the deep layer, suggesting that these cells may be chondrocytes or prechondrocytes. **B′** Deep layer of the lipo-TGF group: positive expression in many chondrocyte-like cells in the deep layer. **A–B′** ×100

the intraarticular retention time of labeled methotrexate could be elongated using liposomes.[8] In the present study, we confirmed that cartilage restoration was accelerated only in the lipo-TGF group, suggesting that this acceleration can be partly explained by the prolonged duration of the intraarticular retention of TGFβ1. The results of our former study — that fluorescence-labeled liposomes tend to accumulate at the site of cartilage repair — suggested one of the reasons liposomal TGFβ1 had significant effects on in vivo cartilage restoration. The results appear to be attributable to the fact that liposomes tend to accumulate at sites of inflammation, possibly owing to the passive targeting property of liposomes.[15,18] Liposome-encapsulated TGFβ1 may thus accumulate at the site of cartilage repair, where it can effectively promote cartilage restoration.

The timing of the intraarticular administration of TGFβ1 appears to be important for cartilage restoration. Shapiro et al. reported that cartilage defects in the superficial to deep layers could be filled with mesenchymal stem cells about 1 week after surgery.[23] Because TGFβ1 could promote differentiation of mesenchymal stem cells into chondrocytes, the intraarticular administration of TGFβ1 1 week after surgery may be effective in promoting cartilage restoration.

Dounchis et al. reported that TGFβ1 had a concentration-dependent effect on cell proliferation in vitro.[6] Our preliminary data on the intraarticular injection of 500 ng TGFβ1, even when encapsulated by liposomes, showed extensive fibroblastic hyperplasia involving the infiltration of inflammatory cells in the synovial membrane and poor cartilage restoration (data not shown): A massive intraarticular injection of TGFβ1 has been reported to induce synovitis[7] and proteoglycan depletion. Moreover, our preliminary data on the intraarticular injection of 10 ng TGFβ1, even when encapsulated by liposomes, showed no difference in histological findings between the controls and the liposome-encapsulated group. It

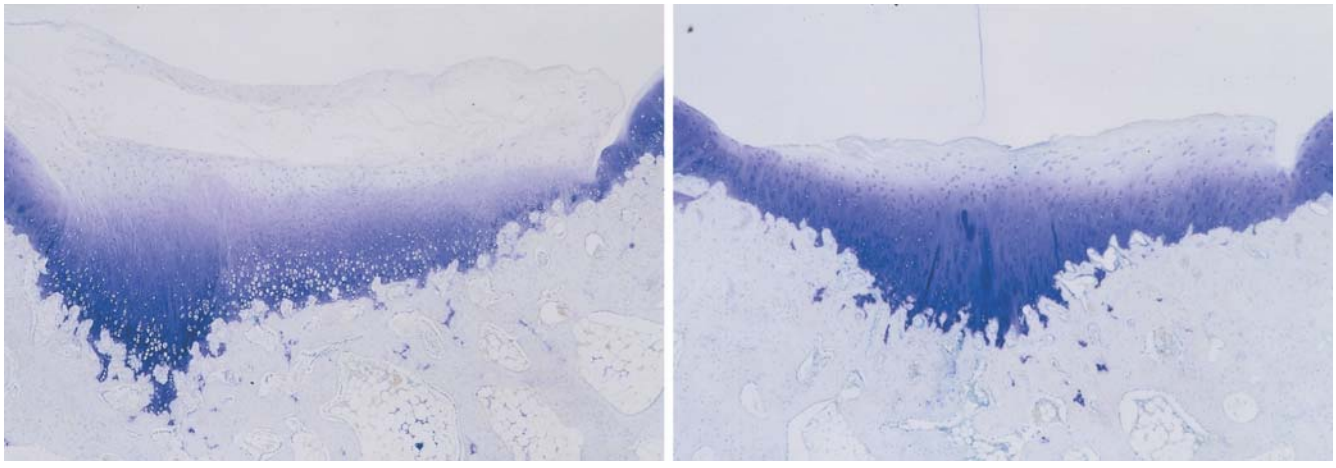

Fig. 6. Histological findings 12 weeks after surgery. **A** PBS group. The defect was filled with a thick, irregular-surfaced repair tissue resembling fibrocartilaginous tissue. The repair tissue showed intense metachromasia only in the deep layer and an irregular extracellular matrix. A nonintegrated gap between the surrounding cartilage and the repair tissue was visible. **B** Lipo-TGF group. The cartilage defect was filled with a thick, irregular-surfaced repair tissue with many round chondrocyte-like cells resembling fibrocartilaginous tissue. The repair tissue showed intense metachromasia in the middle and deep layers and a fine extracellular matrix. A nonintegrated gap between the surrounding cartilage and the repair tissue was also visible. **A,B** toluidine blue ×10

appears that the dose of TGFβ1 is important for cartilage restoration and that TGFβ1 has a positive in vivo influence on chondrogenesis with a suitable range of concentrations.

Our data for the 12-week study showed no significant difference between the controls and the lipo-TGF group in regard to cartilage regeneration. Moreover, our preliminary data at 24 weeks showed that the repair tissue in both groups showed imperfect and degenerative fibrous cartilage (data not shown). These long-term results suggest the limited usefulness of liposomes as a drug delivery system and the difficulty of achieving cartilage restoration by the intraarticular administration of TGFβ1.

Conclusions

The present observations indicate that intraarticular administration of TGFβ1 accelerates the repair of full-thickness articular cartilage defects. This is the first report showing that the intraarticular administration of TGFβ1 effectively promotes cartilage regeneration in a cartilage defect model. Furthermore, the use of liposomes enabled TGFβ1 to act efficiently on cartilage regeneration in the short term. However, our data from a long-term follow-up study suggest that cartilage regeneration is difficult using liposomal TGFβ1. Consequently, more sophisticated drug delivery systems and more effective drugs with no side effects must be developed for perfect cartilage restoration.

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